Temporal and Spatial Variations in Nutrient Stoichiometry and Regulation of Phytoplankton Biomass in Hong Kong waters: Influence of the Pearl River Outflow and Sewage Inputs

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Abstract

In 2001, the Hong Kong government implemented the Harbor Area Treatment Scheme (HATS) under which 70% of the sewage that had been formerly discharged into Victoria Harbor is now collected and sent to Stonecutters Island Sewage Works where it receives chemically enhanced primary treatment (CEPT), and is then discharged into waters west of the Harbor. The relocation of the sewage discharge will possibly change the nutrient dynamics and phytoplankton biomass in this area. Therefore, there is a need to examine the factors that regulate phytoplankton growth in Hong Kong waters in order to understand future impacts. Based on a historic nutrient data set (1986-2001), a comparison of ambient nutrient ratios with the Redfield ratio (N:P:Si=16:1:16) showed clear spatial variations in the factors that regulate phytoplankton biomass along a west (estuary) to east (coastal/oceanic) transect through Hong Kong waters. Algal biomass was constrained by a combination of low light conditions, a rapid change in salinity, and strong turbulent mixing in western waters throughout the year. Potential stoichiometric Si limitation (up to 94% of the cases in winter) occurred in Victoria Harbor due to the contribution of sewage effluent with high N and P enrichment all year, except for summer when the frequency of stoichiometric Si limitation (48%) was the same as P, owing to the influence of the high Si in the Pearl River discharge. In the eastern waters, potential N limitation and N and P co-limitation occurred in autumn and winter respectively, because of the dominance of coastal/oceanic water with low nutrients and low N:P ratios. In contrast, potential Si limitation occurred in spring and a switch to potential N, P and Si limitation occurred in eastern waters in summer. In southern waters, there was a shift from P limitation (80%) in summer due to the influence of the N-rich Pearl River

discharge, to N limitation (68%) in autumn, and to N and P co-limitation in winter due to the dominance of N-poor oceanic water from the oligotrophic South China Sea. Our results show clear temporal and spatial variations in the nutrient stoichiometry which indicates potential regulation of phytoplankton biomass in HK waters due to the combination of the seasonal exchange of the Pearl River discharge and oceanic water, sewage effluent inputs, and strong hydrodynamic mixing from SW monsoon winds in summer and the NE monsoon winds in winter.

Keywords: Stoichiometric nutrient limitation, Pearl River discharge, Sewage effluent, Hong Kong waters, Monsoon

Introduction

Eutrophication of coastal ecosystems is caused by excessive nutrients such as nitrate and phosphate. The increased input of nutrients from riverine outflow and domestic sewage effluent into coastal waters has several ecological consequences: increased algal blooms, the formation of hypoxia or anoxia in the bottom water due to the sedimentation of unused organic matter as stratification develops (Malone et al., 1988; Cooper and Brush, 1991, Welsh and Eller, 1991), and a change in the phytoplankton species composition due to alterations in ambient nutrient ratios and quantities (Jickell, 1998). Usually, N:P and N:Si ratios increase due to high N in fertilizers and in rainfall. An increase in the N:Si ratio has been shown to cause a shift in the dominant siliceous and non-siliceous species (Officer and Ryther, 1980; Fisher et al., 1992). A typical case is in the North Sea, where there had been a dramatic increase in the biomass of flagellates relative to that of diatoms as a result of the increased N:Si ratio in recent decades (Smayda, 1990).

It has been debated which nutrient, N or P, is limiting primary production in the marine environment. Nitrogen has traditionally been considered as the nutrient that limits productivity in coastal waters (Ryther, 1971; Oviatt et al., 1995). Recent studies have shown that P can also be a limiting nutrient in coastal areas associated with periods of high river runoff with high N: P loading ratios (Harrison et al., 1990). In contrast, N, or N+P limitation is linked to low river runoff and a rather greater influence of seawater having a Redfield N:P ratio (Fisher et al., 1992).

Hong Kong is situated on the eastern side of the Pearl River estuary. The coastal waters of Hong Kong are profoundly influenced by 3 nutrient inputs: the N-rich summer

Pearl River discharge, relatively nutrient-poor oceanic waters from the South China Coastal Current, and year round domestic sewage effluent. These water regimes are strongly affected by two seasonal monsoons. In winter when the northeast monsoon prevails, the effect of the Pearl River discharge is minimal as the discharge volume is low, and the South China Coastal Current and oceanic waters with relatively low nutrients dominate the coastal waters of Hong Kong. In summer, when the southwest monsoon prevails and the river discharge is maximal, the Pearl River discharge (with a high N:P ratio) flows into the coastal waters of Hong Kong. Hong Kong discharges > 2 million tons of sewage effluent daily. In 2001, the Hong Kong government implemented the Harbor Area Treatment Scheme (HATS), which collects 70% of the sewage previously discharged into Victoria Harbor, treats it and discharges it into waters two km west near Stonecutters Island. This relocation of sewage effluent is likely to have a significant effect on the dynamics of nutrients and phytoplankton biomass.

However, previous studies have mainly focused on the Pearl River estuary and adjacent areas in summer (Yin et al., 2000, 2001), and more studies are needed to examine Victoria Harbor and the adjacent areas (Yin & Harrison, 2007). Little is known about the impact of the nutrient-rich sewage on the nutrients dynamics and phytoplankton biomass in Hong Kong waters on a seasonal basis. A first step in this regard is to examine the spatial and temporal variability in nutrient stoichiometry over the 15 year period before HATS was implemented in Hong Kong waters and to investigate the implications for nutrient limitation of phytoplankton growth. This will provide a background for comparison with future trends after HATS. Here we describe an analysis of the 15 year (1986-2001) monitoring dataset from the Environmental Protection Department of Hong

Kong, with a focus on the spatial and seasonal variations in nutrients, nutrient ratios and phytoplankton biomass in Hong Kong waters.

Materials and Methods

The Environmental Protection Department (EPD) of the Hong Kong government has maintained a comprehensive sampling program to monitor water quality at >76 monitoring stations in the territorial waters for over 15 years (website: www.epd.gov.hk). Twelve stations located in the western waters (NM2, WM4 and WM3), southern waters (SM9, SM10, SM6 and WM1), Victoria Harbor (VM7, VM5 and VM2) and eastern waters (EM3 and MM8) were selected (Table 1 and Fig. 1). In this paper, only data of 5 representative stations (NM2, WM3, VM5, MM8 and SM6) are presented since the same conclusions on nutrient limitation apply to the other stations (Xu, 2007). These five stations represent the following Hong Kong geographical regions and water quality zones: estuarine influence (NM2, WM3 and SM6), sewage effluents (VM5) and coastal/oceanic conditions (MM8). Monthly or bimonthly sampling was conducted. A SEACAT 19 CTD was used to take vertical profiles of salinity, temperature and other parameters. Water samples were taken at 3 depths: surface, middle and bottom (1 m above the bottom) for chlorophyll *a* (Chl *a*) and nutrients (NO_3, NH_4, NO_2, PO_4, and SiO_4). The analytical methods followed standard methods for the examination of water and wastewater, and are given in the Marine Water Quality Report, Hong Kong (EPD, 1999). Basically, Chl *a* was extracted using acetone and measured spectrophotomerically (APHA, 1995; cf. EPD, 1999). NO_3 and NH_4 were analyzed by the Cu-Cd column reduction method (APHA, 1995; cf. EPD, 1999) and the indophenol blue color formation, respectively (ASTM,

1991; cf. EPD 1999), soluble PO_4 (orthophosphate) by the ascorbic acid method, and SiO_4 by molybdate, oxalic acid and a reducing reagent. TIN (=NO_3+NH_4+NO_2) and PO_4 and SiO_4 concentrations were used to calculate atomic ratios of N:P and N:Si.

Criteria for stoichiometric nutrient limitation

The criteria for stoichiometric nutrient limitation were based on the ambient nutrient ratios (molar ratios). The ratios were used as an indicator of 'potential' nutrient limitation as the actual ambient nutrient concentrations may not be low enough to limit phytoplankton growth. Further tests of nutrient limitation such as nutrient enrichment bioassays and isotope trace techniques were also conducted to confirm the prediction using nutrient ratios of the ambient seawater. The results were consistent with those predicted from ambient nutrient ratios and will be reported elsewhere. The atomic Si:N:P ratio of marine diatoms is about 16:16:1 in a nutrient-replete ecosystem (Redfield, 1958; Brzezinski, 1985). Deviation from the Redfield ratio indicates the potential for N, P, or Si limitation of phytoplankton growth. In our assessment of stoichiometric limitations, we calculated two ambient nutrient ratios for each nutrient and applied the Redfield ratio to predict: (1) that N limitation occurs when N:P <16 and N:Si <1; (2) P limitation occurs when N:P >16 and Si:P >16; and (3) Si limitation occurs when N:Si >1 and Si:P <16. A similar approach was used by Dubravko et al. (1995) in order to assess nutrient limitation in the Mississippi River estuary.

Plots of the atomic Si:P against N:P ratios in the surface waters of Hong Kong indicate which nutrient is potentially limiting for phytoplankton biomass growth. The data points in the upper left quadrant (N:P<16:1) are indicative of N-limitation, in the

upper right quadrant are indicative of P-limitation and of Si-limitation in the lower left quadrant, as shown in Fig. 8. The quadrant with the most data points indicates more frequent occurrences of potential limitation of a particular nutrient.

Statistical analyses

The relationship between chlorophyll *a* and other parameters was analyzed using Sigmaplot and the SPSS program (the Pearson test).

Results

Surface salinity fluctuated seasonally between >30 in December and around 15 in July at NM2 (Fig. 2). At MM8, salinity was >27 all year. In summer (June to August), stratification of the water column was observed at all stations, accompanied by an increase in surface salinity along the transect from the western estuarine waters (NM2) to eastern coastal/oceanic waters (MM8). In southern waters (SM6), the lowest salinity was ~ 21 in summer, indicating significant influence from the Pearl River estuary. In winter (December to February), salinity between surface and bottom at all 5 stations was almost uniform due to vertical mixing and remained >30. Temperature at the surface showed a seasonal fluctuation between high (24 to 29℃) in summer and low (15 to 21℃) in winter at all stations (Fig. 2).

There was a seasonal change in the surface NO_3 concentration at all stations with a maximum in summer and a minimum in winter (Fig. 3). There was also a spatial gradient of NO_3 during summer: NO_3 decreased along the west (NM2) to east (MM8) transect. Monthly peak NO_3 concentrations were around 60 μM at NM2 during July (Fig. 3). In

contrast, NO_3 concentrations at the surface and bottom were relatively low at MM8, with a maximum value of only 10 um in June (Fig. 3). The seasonal pattern in SiO_4 was very similar to NO_3 at all stations since both NO_3 and SiO_4 inputs were mainly from the Pearl River (Fig. 3). NH_4 concentration was higher in Victoria Harbor (VM5) than the other stations (Fig. 4). There was a seasonal change in NH_4 concentration with the lowest value during June and July at WM3 and VM5 probably due to phytoplankton utilization. PO_4 concentrations exhibited a similar pattern to NH_4 at WM3 and VM5, with the lowest concentrations in the summer and high (>1 μM) in the dry seasons. At the other three stations (NM2, MM8 and SM16), no clear seasonal variability in both NH_4 and PO_4 was observed.

Chl *a* concentrations were relatively low (<3 μg L^{-1}) at most stations during the dry season and reached a peak in July at all stations (Fig. 5). In summer, the maximum Chl *a* (~ 15 μg L^{-1}) occurred at VM5 and SM6. At NM2, Chl *a* concentration was near or < 5 μg L^{-1} all year (Fig. 5).

The N:P ratios also exhibited seasonal fluctuations, with low ratios in winter and high ratios in summer (Fig. 6). At NM2, the N:P ratios at the surface were 24:1 during the dry season (October to March) and increased to >48:1 during the wet season (April to September). At WM3, VM5 and SM6, the N:P ratios were close to the 16:1 during the dry season and the maximum value occurred (~ 48:1 at WM3 and VM5, >80:1 at SM6) in summer (Fig. 6). A surprisingly low ratio (5:1) was observed at SM6 in May (Fig. 6). N:P ratios were < 16:1 during autumn (September to November) at MM8, with a maximum value of 40:1 in June.

Seasonal patterns of the Si:P ratios were similar to N:P ratios at the surface (Fig. 6). The Si:P ratios were > 16:1 all year at NM2, MM8 and SM6. At VM5, the Si:P ratios were low (<16:1) at all times, except in summer when Si:P ratios ranged from 24 to 43 (Fig. 6).

The N:Si ratios varied spatially and seasonally (Fig. 7). The N:Si ratios were above or close to 1:1 at NM2 and > 1:1 at WM3 and VM5 at all times. There was clear temporal variations in the N:Si ratios at the surface at MM8 high(>1:1) in summer and low (<1:1) during the dry season. At SM6, the N:Si ratios were generally < 1:1 all year except in June and August (Fig. 7).

Surface suspended solids (SS) also exhibited the spatial and seasonal variations (Fig. 7). In general, SS were higher (near or > 5 mg L^{-1}) at NM2, WM3 and VM5, and lower (<5 mg L^{-1}) at MM8 and SM6 (Fig. 7). Seasonal variations were observed at NM2 and WM3 with higher concentrations in winter and lower in summer (Fig. 7).

The atomic N:P, N:Si and Si:P ratios for surface Hong Kong waters exhibited spatial and seasonal variations in stoichiometric nutrient limitation (Figs. 8 and 9). During summer, the probability of stoichiometric P limitation decreased along the west (>95% at NM2) to east transect (~ 30% at MM8). There were equal percentages of both stoichiometric P (48%) and Si (48%) limitation at VM5 in the summer (Figs. 8 and 9). At MM8, stoichiometric N, P and Si limitation appeared to be equally likely (30% N, 30% P and 40% Si) during summer (Figs. 8 and 9). From summer to winter, stoichiometric P limitation decreased in Hong Kong waters except for eastern waters, accompanied by an increase in stoichiometric N and Si limitation (Figs. 8 and 9). There was a seasonal switch in stoichiometric nutrient limitation at SM6 and WM3, from potential N limitation

(>50%) in autumn and winter to P limitation (~ 53%) in spring and summer at SM6, and from potential Si limitation (>58%) in winter and spring to P limitation (>64%) in summer and autumn at WM3 (Figs. 8 and 9).

Discussion

Seasonal alteration of freshwater outflow and coastal/oceanic water

Strong seasonal changes in salinity, temperature, Chl *a*, and nutrients are coupled with seasonal monsoons and the Pearl River discharge (Yin, 2002). During the winter dry season, when the Pearl River discharge is low (3,343 $m^3 s^{-1}$, Zhao, 1990), the NE monsoon winds and the Coriolis force move the estuarine water to the west side of the estuary and consequently there is little contribution of the Pearl River to Hong Kong waters. However, the winter NE monsoon winds advect coastal/shelf surface water with relatively high salinity and low nutrients into Hong Kong waters (Yin, 2002). Hong Kong is dominated by the China Coastal Current from the northeast in winter (Watts, 1973; Yin et al., 1999), and salinity is high at the surface and the water column is mixed homogeneously during this period.

In summer, Hong Kong experiences the south-southwest monsoon (Yin, 2002), and the Pearl River discharge reaches a maximum (22,190 $m^3 s^{-1}$) in July (Zhao, 1990). The Pearl River estuary is dominated by the typical two-layer estuarine circulation with the outflow of the estuarine plume at the surface and a typical salt wedge inflow in the bottom layer (Yin et al., 2001). The estuarine plume flows out of the estuary, forming the coastal plume and it spreads out over the whole study area, as indicated by decreased salinity at the surface in summer at all stations (Fig. 2). Both the coastal upwelling

resulting from the SW monsoon and to the Coriolis effect along the coast and the outflow of the Pearl River drive the offshore movement of the estuarine coastal plume (Watts, 1973; Yin, 2002). As a result, the deep oceanic water on the continental shelf moves shoreward at the bottom to compensate for the surface outflow (Chau and Wong, 1960; Yin, 2002). Therefore, in summer, the bottom water comes from the continental shelf, as indicated by high salinity (>30) and low temperature as well as relatively low nutrients at most stations except for NM2. At the shallow station (NM2), vertical mixing of low salinity surface water induced by winds and tides resulted in a decrease in salinity at the bottom in summer (Fig. 2).

Seasonal and spatial variations in factors regulating phytoplankton biomass

Western region

Western waters are strongly impacted by the Pearl River discharge in summer, the period of the maximum rainfall and Pearl River discharge. Nutrient ratios indicated that stoichiometric P limitation dominated in the western waters all year, especially in summer, the period of high N inputs due to the maximum rainfall and Pearl River discharge (Figs. 8 and 9). Nevertheless, the expected algal blooms are not found in this region in spring and summer, in spite of high nutrients. Therefore, physical processes may play an important role in regulating the phytoplankton biomass.

A mixing diagram of a nutrient concentration vs salinity along an estuary for a given time interval can often be indicative of a sink (removal) or a source (release) of the nutrient. Similarly, the same diagram using data over many years can be indicative of the stability of two end members. When a significant relationship between a nutrient and

salinity exists, it may suggest that there have been no significant changes in the two end members of the nutrient. Since oceanic nutrients are very low and changes exert a small influence on estuarine waters, it is the nutrient in the freshwater water end member that influences the mixing diagram the most. The significant correlations between salinity and NO_3 or SiO_4 suggest that the freshwater source of NO_3 and SiO_4 has not changed significantly and both nutrients behave nearly conservatively at NM2 in the last 15 years (Table 2, Fig 10). NO_3 and SiO_4 concentrations in freshwater from the Pearl River estimated by the intercept concentrations, are similar to the observed values (NO_3 75-100 μM, SiO_4 130-140 μM) at the near-zero salinity end-member in the Pearl River estuary (Yin et al., 2000, 2001; Cai et al., 2004). Three-dimensional hydrodynamic model calculations indicated that peak surface current velocities of up to 2 m s^{-1} in this region are due to the narrow channels (Lee et al., 2006). These findings indicate that biological utilization of NO_3 and SiO_4 was insignificant during transport from the Pearl River to the west of Hong Kong, possibly due to light limitation induced by the strong vertical turbulent mixing (transporting the phytoplankton out of the photic zone). This hypothesis is supported by the observation of no significant net carbon release or uptake in most of the Pearl River estuary (Cai et al., 2004). Therefore, the accumulation of phytoplankton biomass is probably constrained by a combination of low light conditions, rapid changes in salinity, and strong turbulent mixing. This result explains why low Chl *a* and high NO_3 and SiO_4 occurred at NM2 during summer. In other systems with high advective throughput, nutrient concentrations are also found to be nearly conservative with respect to non-reactive tracers such as salinity (Boyle et al., 1974; Biggs and Cronin, 1981; Schindler, 1981). Imberger et al. (1983) pointed out that the near-conservative behavior

of nitrogen and phosphorus with respect to salinity indicated that nutrient concentration was controlled primarily by physical processes of advection and mixing, and not by net biological uptake.

When nutrient data from many years are combined and plotted against salinity, the yearly signal (variability) is smeared. Therefore, the mixing diagram of a nutrient against salinity was plotted for each year in order to determine interannual variability. If the relationship between a nutrient and salinity is significant, the intercept may be used to indicate the source (end member) concentration of the nutrient. At NM2, the intercepts showed an increasing trend in NO_3 concentration, but not in SiO_4 concentrations over 15 years (Fig. 11). This may suggest that the NO_3 input concentration has gradually increased over the years, though there has, of course, been interannual variability as well. The variability may not be sufficient to change the significant relationship between NO_3 and salinity at NM2.

Victoria Harbor

High NH_4 and PO_4 levels are attributed to the input of sewage effluent in Victoria Harbor, which leads to stoichiometric Si deficiency relative to N and P. Therefore, stoichiometric Si limitation occurred in the dry season when the coastal/oceanic water dominated. In contrast, in spring, when the river discharge with high Si begins to influence this region, the frequency of stiochiometric Si limitation declined, accompanied by an increase in stoichiometric P limitation. This trend became more obvious during the spring to summer transition period. An alteration of stoichiometric Si and P limitation was observed due to the contribution of the Pearl River discharge with high N:P ratios

during summer, the period of highest rainfall, when both NH_4 and PO_4 reached a minimum due to utilization by phytoplankton. This is probably due to the development of high biomass blooms in the stratified waters under low wind conditions and high temperature (~30°C).

Over the monitoring period, sewage discharges into Victoria Harbour received only preliminary treatment; sewage after screening and degritting were discharged into the harbor through a number of submarine outfalls that aim to achieve an initial dilution of around 100. Thus the water quality in Victoria Harbor reflects that of a highly diluted (mixing by the tidal flow) sewage effluent. The regression equation for surface NH_4 to PO_4 concentrations revealed that N:P in the diluted sewage effluent was about 10:1 (Fig. 12). This is less than the Redfield ratio of 16:1, suggesting that P was in excess relative to N in sewage effluent since NH_4 is considered to be the main N source since it is preferentially utilized over NO_3. The relationships between Chl *a* and nutrients (TIN, NH_4, PO_4 and SiO_4) are revealing: First, a significant region of high nutrient and low Chl *a* (to the right end of each curve) can be noted; this is indicative of light limitation due to strong vertical mixing. Second, the relatively high biomass data can be roughly fitted by an exponential decay equation (Fig. 13). Derived from the regression equations, the potential maximum Chl *a* concentrations that could be supported by TIN, NH_4, PO_4 and SiO_4, respectively, were estimated to be 40, 25, 30, 24 $\mu g\ L^{-1}$ (Fig. 13) when these nutrients were fully utilized ($x = 0$), assuming the other nutrients were not limiting. The potential maximum Chl *a* value (25 $\mu g\ L^{-1}$) for NH_4 was lower than for PO_4, suggesting that N was the potential limiting nutrient in the sewage, in agreement with the low N:P ratio (~ 10:1) from the regression of NH_4 vs PO_4.

Similarly, the potential maximum Chl *a* (24 μg L^{-1}) for Si was found to be less than N and P in seawater, indicative of potential Si limitation, and in agreement with the results inferred from the ambient nutrient ratios (Fig. 8 and 9). The input of the freshwater added more NO_3 to Victoria Harbor, which resulted in an increase in N:P and N:Si ratios. Therefore, Si was stoichiometrically the potentially limiting nutrient in Victoria Harbor. The low biomass in Victoria Harbor is possibly due to the influence of environmental factors such as vertical mixing and grazing. For example, when a water sample from which large zooplankton were removed using a 200 μm mesh net was incubated in bottles under natural sunlight, Chl *a* reached a maximum concentration of 52 μg L^{-1} in the control treatment (no nutrient addition) (Xu, 2007). Victoria Harbor is strongly affected by tidal mixing, as indicated by the small differential between surface and bottom salinity and low bottom salinity compared to WM3 and MM8 (Fig. 2). In addition, chlorophyll was nearly 5 μg L^{-1} in the bottom water at VM5 (Fig. 5), even though the bottom waters are well below the photic zone. This observation is further evidence for vertical mixing in Victoria Harbor, in agreement with observations by Yin and Harrison (2007). The flushing time was reported to be 1.5-2.5 days in the wet season (Lee et al., 2006). The stratification is weak and short-lived due to the strong hydrodynamic vertical mixing and horizontal transport, which reduces the eutrophication effect and explains the reduced occurrence of algal blooms in Victoria Harbor (Yin, 2002; Wong et al., 2007). The resistance of a coastal ecosystem to increasing eutrophic conditions has also been observed in the Bay of Brest due to the high hydrodynamic mixing and increased secondary production in the benthos (Pape et al., 1996).

Eastern waters

The eastern waters are relatively sheltered from the Pearl River discharge and hence they have more oceanic characteristics with relatively high surface salinity (Yin, 2002; Lee et al., 2006). In this region, N appeared to be the most limiting nutrient, except for spring when Si was the potentially limiting. In winter, the period of dominance by coastal/oceanic water, N and P co-limitation was observed, possibly due to the nearly balanced N:P ratios in coastal/oceanic water. In summer, a switch in stiochoimetric N, P and Si limitation occurred, and depended on the extent of the influence of the Pearl River discharge. N limitation at high salinity has also been previously reported for coastal waters impacted by the Mississippi River plume (Lohrenz et al., 1999).

Southern waters

During the summer period of highest Pearl River discharge, the peak Chl *a* value of 33 μg L^{-1} was recorded (Fig. 5). High phytoplankton biomass forms downstream, especially at the edge of the coastal plume. The plume frontal regions have also been found to have high Chl *a* concentrations relative to inshore and offshore waters in other coastal areas such as the Strait of Georgia (Yin et al., 1997), Chesapeake Bay (Breitburg, 1990; Harding, 1994) and the Mississippi River plume (Grimes and Finucane, 1991). The relatively high Chl *a* level may be associated with waters that have a longer residence time in the coastal plume due to increased stratification. An obvious seasonal variability in stoichiometric nutrient limitation was demonstrated in the southern waters, characteristic of stoichiometric N, or N+P co-limitation in winter and stoichiometric P limitation in summer. In summer, the Pearl River discharge covers the whole southern

waters region, with low salinity and high NO_3 concentration at the surface. Hence, stoichiometric P limitation occurred frequently in our analysis. In winter, the nutrient-poor coastal/oceanic waters dominated in this region, characterized by low surface NO_3 and high salinity. As a result, potential stoichiometric N or N+P co-limitation occurred.

In this study, we did not measure organic and total nutrients; however, the effect on the estimation of nutrient ratios for the study of phytoplankton dynamics is expected to be insignificant. In general, phytoplankton biomass are more dependent on dissolved inorganic than organic nutrients although organic nutrients may be important for some species. Furthermore, only a portion of the dissolved organic nutrients is likely to be bioavailable (Bronk et al., 1994). In addition, the inorganic nitrogen due to the sewage input in Hong Kong is contributed mainly by ammonia nitrogen. For example, based on the nutrient concentrations in the sewage effluent, it can be estimated that the organic nitrogen input contributes to only around 10 percent of the ammonia nitrogen in Victoria Harbour. Moreover, the part of organic nitrogen that is hydrolysed (to ammonia nitrogen) is already accounted for in the observed inorganic nitrogen concentrations. This study shows that in complex estuarine waters, the algal biomass depends not only on the total nutrient loading or the N/P ratios, but to a great extent also determined by physical factors.

There were the effects of nutrient limitation on dynamics of phytoplankton groups during the study period. This topic will be covered in another paper (Ho et al., submitted). In general, phytoplankton biomass and production in HK waters were dominated by the chain-forming diatoms (>5 μm) in summer, but dominance of dinoflagellates increases in winter, probably because of relatively high Si input from the PRE (Ho et al., submitted).

In summary, the factors regulating phytoplankton biomass are complex in Hong Kong waters. The seasonal alteration of the Pearl River discharge and coastal/oceanic water intrusions induced by the monsoon, in addition to sewage effluent input, zooplankton grazing, and physical forcings (e.g., vertical mixing) play an important role in the spatial and temporal variations in nutrients and phytoplankton biomass in Hong Kong waters. In western waters, phytoplankton growth was primarily limited by the rapid dilution and possible light limitation due to strong vertical turbulent mixing , which together reduce the impacts of eutrophication. In Victoria Harbor, a seasonal shift was observed from potential Si limitation in autumn and winter under the dominance of the coastal/oceanic water to P+Si co-limitation in spring and summer owing to the influence of the Pearl River discharge. The short flushing time in the wet season and strong hydrodynamic mixing hinder the accumulation of phytoplankton biomass. In eastern waters, potential N limitation was more likely to occur particularly in the dry season. In the southern waters, a shift from P limitation in summer to N limitation in winter occurred due to the seasonal exchange of the Pearl River discharge and coastal/oceanic water.

During the winter dry season, the relatively low Chl *a* concentrations can be attributed to strong vertical mixing which can transport phytoplankton cells to depth, reducing light availability for phytoplankton growth, which also occurs due to the relatively high SS at that time (Fig. 13). Victoria Harbor is a typical example of this with high nutrients and low Chl *a* in winter. This suggestion is supported by the significant negative correlation between the monthly average Chl *a* and monthly average suspended solids in the western regions and Victoria Harbor (Fig. 14). This effect can be enhanced

by strong winds and/or heavy navigation traffic through the narrow harbor channel (Yin, 2002). Light limitation has also been reported in coastal areas of the eastern USA in winter (Fisher et al., 1999).

The results from this research provide information on phytoplankton stoichiometric limitation in the coastal waters of Hong Kong and can assist in the development of sewage strategies. Seasonal variations in the factors that regulate phytoplankton biomass suggest the possibility of adopting more economic and adaptive seasonal management strategies for nutrient removal. In summer, the removal of P should be the primary consideration in sewage treatment. Furthermore, the cost of P removal is much lower than N. In contrast, the Hong Kong coastal ecosystem is more resistant to the eutrophication impacts in winter due to the strong hydrodynamic mixing, and hence, nutrient removal is much less important.

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Figure Legends

Fig. 1. The location of the sampling stations in Hong Kong waters. These 12 stations are the same as the EPD stations. They represent a gradient from western eutrophic waters (NW2) to eastern waters with much lower nutrients (MM8).

Fig. 2. Monthly average salinity and temperature at the surface and bottom at 5 stations from 1986-2001. Vertical bars indicate ±1 SD. (Data from EPD, Hong Kong).

Fig. 3. Monthly average NO_3 and SiO_4 at the surface and bottom at 5 stations from 1986-2001. Vertical bars indicate ±1 SD. Note the different concentration scale for different stations. (Data from EPD, Hong Kong).

Fig. 4. Monthly average NH_4 and PO_4 at the surface and bottom at 5 stations from 1986-2001. Vertical bars indicate ±1 SD. Note the different concentration scale for different stations. (Data from EPD, Hong Kong).

Fig. 5. Monthly average TIN (=NO_3+NH_4+NO_2) e surface and bottom at 5 stations from 1986-2001. Vertical bars indicate ±1 SD. Note the different concentration scale for different stations. (Data from EPD, Hong Kong).

Fig. 6. Monthly average N:P and Si:P ratios at the surface and bottom at 5 stations from 1986-2001. Vertical bars indicate ±1 SD. The horizontal dashed line indicates the Redfield ratios of 16:1 for N:P and Si:P. (Data from EPD, Hong Kong).

Fig. 7. Monthly average N:Si ratios and suspended solids (SS) at the surface and bottom at 5 stations from 1986-2001. Vertical bars indicate ±1 SD. The horizontal dashed line indicates a Redfield N:Si ratio of 1:1. (Data from EPD, Hong Kong).

Fig. 8. Scatter diagrams of atomic nutrient ratios for the surface water during spring (Δ, Mar to May), summer (□, Jun to Aug), autumn (●, Sep to Nov) and winter (○, Dec to

Feb); N, P and Si represent dissolved inorganic N, P, and Si. Stoichiometric (= potential) limitation for N, P, and Si is indicated by the number of data points in the various quadrants (Data from EPD, Hong Kong).

Fig. 9. Potential (%) N, P or Si limitation determined from plots in Fig. 8 for surface waters during spring (March to May), summer (June to August), autumn (September to November) and winter (December to February) at five stations in Hong Kong Waters from 1986 to 2001 (Data from EPD, Hong Kong).

Fig. 10. Concentrations of NO_3 and SiO_4 versus salinity for the surface data for five stations from the time series from 1986 to 2001. The graphs with lines indicate a significant correlation between the two variables and the correlation coefficients are represented by r. The intercept of the regression line is represented by b (Data from EPD, Hong Kong).

Fig. 11. Intercept concentrations of NO_3 and SiO_4 at surface at NM2 from 1986 to 2001. $p_{NO3} < 0.05$ and $p_{SiO4} > 0.05$, indicates that the correlation is significant for NO_3, not SiO_4 at the 0.05 level (2- tailed test) (Data from EPD, Hong Kong).

Fig. 12. NH_4 versus PO_4 at VM5 for the surface concentrations from the time series from 1986-2001. The regression equation of NH_4 to PO_4 and the correlation coefficients r are given. (Data from EPD, Hong Kong).

Fig. 13. Chl *a* concentration versus nutrients (TIN, NH_4, PO_4 and SiO_4) at VM5 for the surface data from the time series from 1986-2001 (Data from EPD, Hong Kong). The regression equation of NH_4 to PO_4 and the correlation coefficients r are given.

Fig. 14. Monthly average Chl *a* versus monthly average suspended solids (SS) for the surface data at NM2 and VM5. The correlation coefficients, r, are given (Data from EPD, Hong Kong).

References

APHA (American Public Health Association), 1995. Standard methods for the examination of water and wastewater: including bottom sediments and sludges. American Public Health Association, New York.

ASTM (American Society for Testing and Materials), 1991. Annual book of ASTM standards. ASTM, Philadelphia.

Biggs, E.R., Cronin, L.E., 1981. Special characteristics of estuaries,. In Neilson, B.J. Cronin L.E. (Eds.) Estuaries and nutrients. Humana, p. 3-23.

Boyle, E., Collier, R., Dengler A.T., Edmond, J.M., Ng, A.C., Stallard, R.F., 1974. On the chemical mass balance in estuaries. Geochimica. Cosmochimica. Acta 38, 1719-1728.

Breitburg, D.L., 1990. Near-shore hypoxia in the Chesapeake Bay: patterns and relationships among physical factors. Estuarine, Coastal and Shelf Science 30, 593-609.

Bronk, D.A., Glibert, P.M., Ward, B.B., 1994. Nitrogen uptake, dissolved organic nitrogen release, and new production. Science 265, 1843-1846.

Brzezinski, M.A., 1985. The Si:C:N ratio of marine diatoms: interspecific variability and the effect of some environment variables. Journal of Phycology 15, 305-311.

Cai, W.J., Dai, M.H., Wang, Y.C., Zhai, W.D., Huang, T., Chen, S.T., Zhang, F., Chen, Z.Z., Wang, Z.H., 2004. The biogeochemistry of inorganic carbon and nutrients in the Pearl River estuary and adjacent Northern South China Sea. Continental Shelf Research 24, 1301-1319.

Chau, Y.K., Wong, C.S., 1960. Oceanographic investigations in the northern region of the South China Sea off Hong Kong. Hong Kong University Fish Journal 3, 1-25.

Cooper, S., Brush, G., 1991. Long-term history of Chesapeake Bay anoxia. Science 254, 992-996.

Dubravko, J., Rabalais, N.N., Turner, R.E., Dortch, Q., 1995. Changes in nutrient structure of river-dominated coastal waters: stoichiometric nutrient balance and its consequences. Estuarine, Coastal and Shelf Science 40, 339-356.

EPD (Environmental Protection Department), 1999. Marine water quality in Hong Kong in 1998: results for 1998 from the Marine Monitoring Programme of the Environmental Protection Department. Hong Kong SAR Government, Hong Kong.

Fisher, T.R., Peele, E.R., Ammerman J.W., Harding L.W. Jr., 1992. Nutrient limitation of phytoplankton in Chesapeake Bay. Marine Ecology Progress Series 82, 51-63.

Fisher, T.R., Gustafson, A.B., Sellner, K., Lacouture, R., Haas, L.W., Wetzel, R.L., Magnien, R., Everitt, D., Michael, B., Karrch, R., 1999. Spatial and Temporal variation of resource limitation in Chesapeake Bay. Marine Biology 133, 763-778.

Grimes, C.B., Finucane, J.H., 1991. Spatial distribution and abundance of larval and juvenile fish, chlorophyll, and macrozooplankton around the Mississippi River discharge plume, and the role of the plume in fish recruitment. Marine Ecology Progress Series 75, 109-119.

Harrison, P.J., Hu, M.H., Yang, Y.P., Lu, X., 1990. Phosphate limitation in estuarine and coastal waters of China. Journal of Experimental Marine Biology and Ecology 140, 79-87.

Harding, L.W. Jr., 1994. Long-term trends in the distribution of phytoplankton in Chesapeake Bay: role of light, nutrients and streamflow. Marine Ecology Progress Series 104, 267-291.

Ho, A.Y.T., Xu, J., Yin, K.D., Jiang, Y.L., Yuan, X.C., He, L., Anderson, D.M., Lee, J.H.W., Harrison, P.J., Seasonal variation in size-fractionated phytoplankton biomass and production along a eutrophic gradient in Hong Kong waters. Submitted to Marine Ecology Progress Series.

Imberger, J., Berman, T., Christian, R.R., Sherr, E.B., Whitney, D.E., Pomeroy, L.R., Wiegert, R.G., Wiebe, W.J., 1983. The influence of water motion on the distribution and transport of materials in a salt marsh estuary. Limnology and Oceanography 28, 201-214.

Jickells, T.D., 1988. Nutrient biogeochemistry of the coastal zone. Science 281, 217-222.

Lee, J.H.W., Harrison, P.J., Kuang, C., Yin, K.D., 2006. Eutrophication dynamics in Hong Kong coastal waters: physical and biological interactions. In Wolanski E. (Eds.), The Environment in Asia Pacific Harbour. Springer. Printed in the Netherland, p. 187-206.

Lohrenz, S.E., Fahnenstiel, G.L., Redalje, D.G., Lang, G.A., Dagg, M.J., Whitledge, T.E., Dortch, Q., 1999. Nutrients, irradiance, and mixing as factors regulating primary production in coastal waters impacted by the Mississippi River plume. Continental Shelf Research 19, 1113-1141.

Malone, T.C., Crocker, L.H., Pike S.E., Wendler, B.W., 1988. Influences of river flow on the dynamics of phytoplankton production in a partially stratified estuary. Marine Ecology Progress Series 48, 235-249.

Officer, C.B., Ryther, J.H., 1980. The possible importance of silicon in marine eutrophication. Marine Ecology Progress Series 3: 83-91.

Oviatt, C., Doering, P., Nowicki, B., Reed, L., Cole, J., Frithsen, J., 1995. An ecosystem level experiment on nutrient limitation in temperate coastal marine environments. Marine Ecology Progress Series 116, 171-179.

Pape, L.O., Amo, Y.D., Menesguen, A., Aminot, A., Quequiner, B., Treguer, P., 1996. Resistance of a coastal ecosystem to increasing eutrophic conditions: the Bay of Brest (France), a semi-enclosed zone of Western Europe. Continental Shelf Research 16, 1885-1907.

Redfield, A.C., 1958. The biological control of chemical factors in the environment. American Science 46, 205-221.

Ryther, J.H., Dunstan, W.M., 1971. Nitrogen, phosphorus and eutrophication in the coastal marine environment. Science 171, 1008-1013.

Schindler, D.W., 1981. Special of eutrophication in lakes and their relevance to the estuarine environment. In: Neilson B.J. and Cronin L.E. (Eds.), Estuaries and nutrients. Humana, p. 71-82.

Smayda, T.J., 1990. Novel and nuisance phytoplankton blooms in the sea: evidence for a global epidemic. In: Graneli, E., Sundstroem, B., Edler, L., Anderson, D.M. (Eds), Toxic Marine Phytoplankton. Elsevier. New York, pp. 29-40.

Watts, J.C.D., 1973. Further observations on the hydrology of the Hong Kong territorial waters. Hong Kong Fisheries Bulletin 3, 9-35.

Welsh, B.L., Eller, F.C., 1991. Mechanisms controlling summer time oxygen depletion in western Long Island Sound. Estuaries 14, 265-278.

Wong, K.T.M., Lee, J.H.W., Hodgkiss, I.J., 2007. A simple model for forecast of coastal algal blooms. Estuarine, Coastal and Shelf Science 74, 175-196.

Xu, J., (2007) Nutrient limitation in the Pearl River estuary, Hong Kong waters and adjacent South China Sea waters. PhD thesis, The Hong Kong University of Science and Technology, Hong Kong.

Yin, K.D., Harrison, P.J., St. John, M.A., Beamish, R.J., 1997. Effects of a fluctuation in Fraser River discharge on primary production in the central Strait of Georgia, British Columbia, Canada. Canadian Journal of Fisheries and Aquatic Science 54, 1015-1024.

Yin, K.D., Harrison, P.J., Chen, J.C., Huang, W., Qian, P.Y., 1999. Red tides during spring 1998 in Hong Kong: is El Niño responsible? Marine Ecology Progress Series 187, 289-294.

Yin, K.D., Qian, P.Y., Chen, J.C., Hsieh, D.P.H., Harrison, P.J., 2000. Dynamics of nutrients and phytoplankton biomass in the Pearl River estuary and adjacent waters of Hong Kong during summer: preliminary evidence for phosphorus and silicon limitation. Marine Ecology Progress Series 194, 295-305.

Yin, K.D., Qian, P.Y., Wu, M.C.S., Chen, J.C., Huang, L.M., Song, X.Y., Jian, W.J., 2001. Shift from P to N limitation of phytoplankton biomass across the Pearl River estuarine plume during summer. Marine Ecology Progress Series 221, 17-28.

Yin, K.D., 2002. Monsoonal influence on seasonal variations in nutrients and phytoplankton biomass in coastal waters of Hong Kong in the vicinity of the Pearl River estuary. Marine Ecology Progress Series 245, 111-122.

Yin, K.D., Harrison, P.J., 2007. Influence of the Pearl River estuary and vertical mixing in Victoria Harbor on water quality in relation to eutrophication impacts in Hong Kong waters. Marine Pollution Bulletin 54, 646-656.

Zhao, H., 1990. Evolution of the Pearl River estuary. Ocean Press, Beijing (in Chinese).